Prediction of human toxicity from animal data

18/31 (58%) positive in animal (R:10, NR:16, R+NR:8)

13/31 (42%) negative in animal

31/238 (13%) cases of hepatotoxicity observed in drug development

14/31 (45%) terminated*

17/31 (55%) continued

*14/84 (17%) compounds terminated due to hepatotoxicity (Unpublished data, ILSI project 1999)

High Priority Initiative from 2001 Workshop

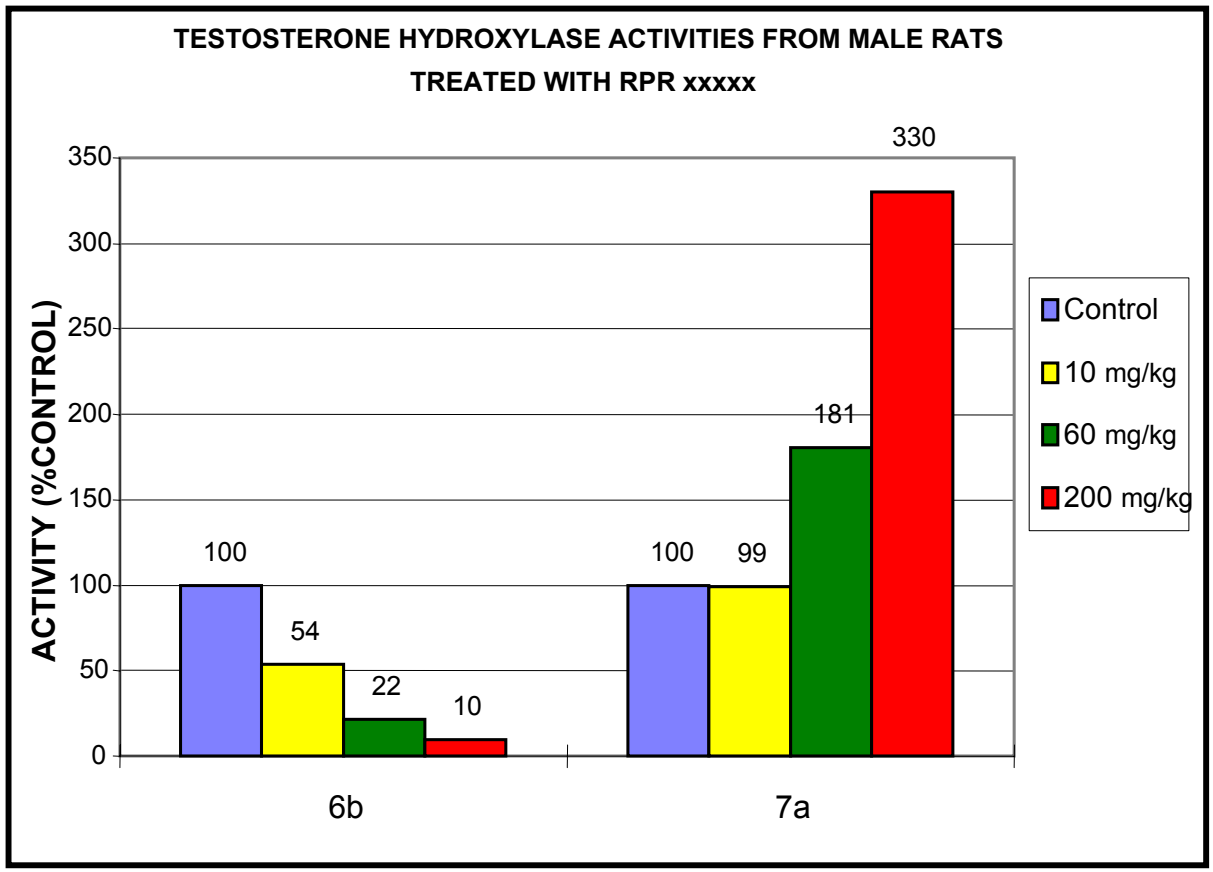
- **Update progress made in the testing for hepatotoxicants**
- **Suggested to approach outside group for implementation (ILSI/HESI specifically named)**

Advances with Regard to Liver Toxicity

- Genomics, proteomics, metabonomics
- Drug Metabolism
  - Adduct formation
  - Reactive intermediates
  - Mechanism based inhibition
- Inflammation
  - Cytokines *TNFa, IL-1…*
  - Chemokines *IL-8, MIP-1, MIP-2…*
  - Cytotoxic factors *reactive oxygen species, cytolytic proteases, nitric oxide…*

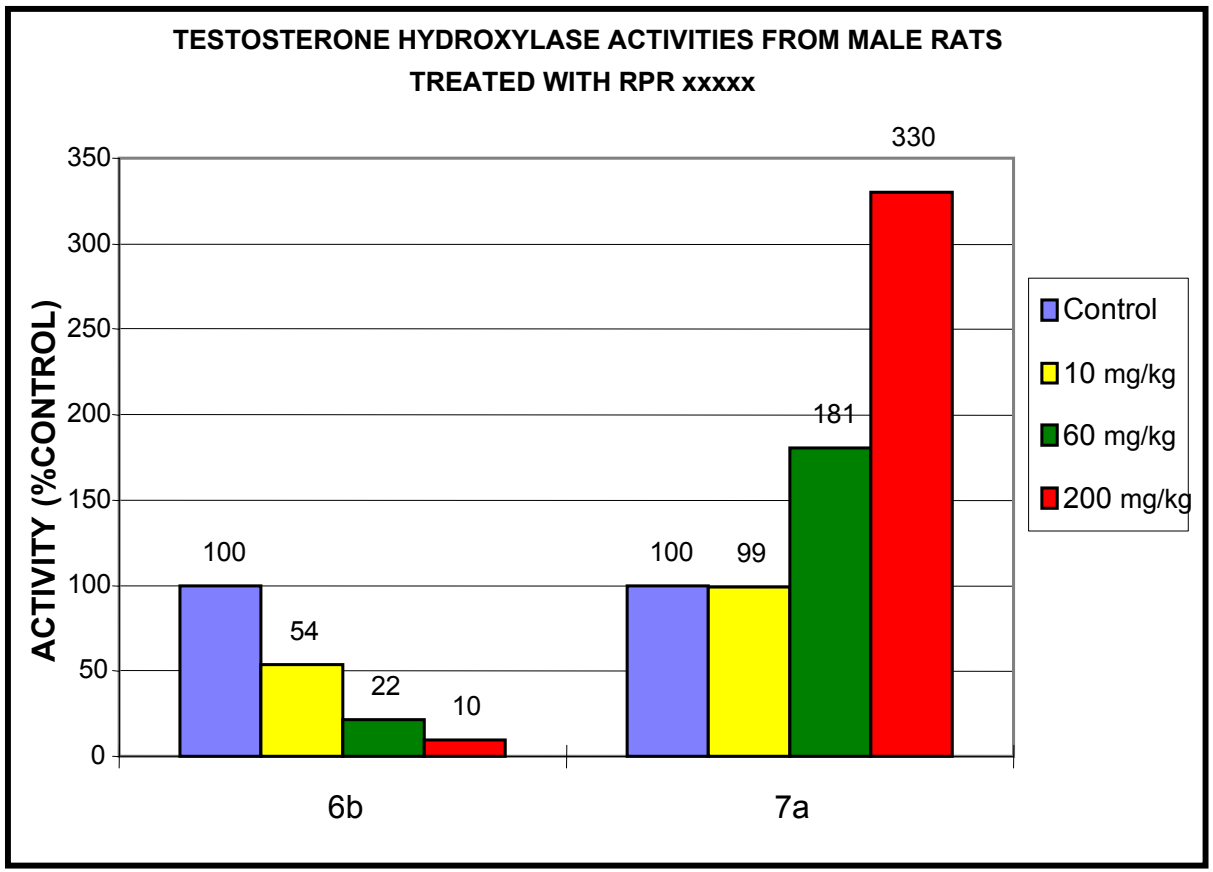
Mechanism-based inhibition (HPLC).

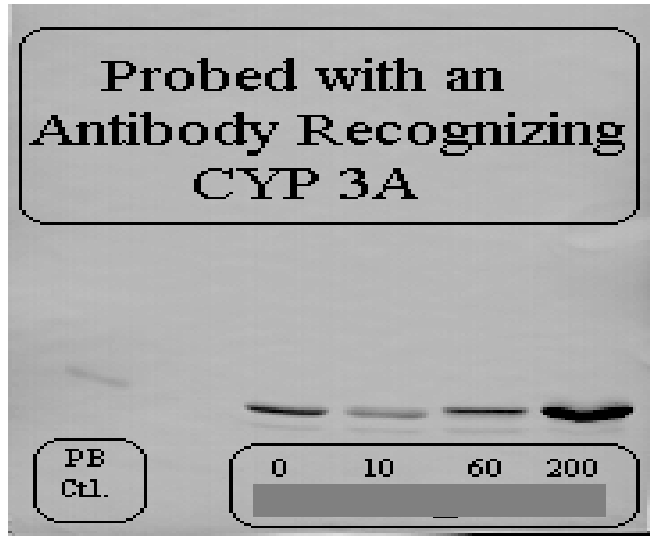
RPR xxxxx: 14-DAY BID ORAL EXPLORATORY TOXICITY STUDY IN RATS



Decrease in protein activity corresponding with an induction at the protein level

RPR xxxxx: 14-DAY BID ORAL EXPLORATORY TOXICITY STUDY IN RATS

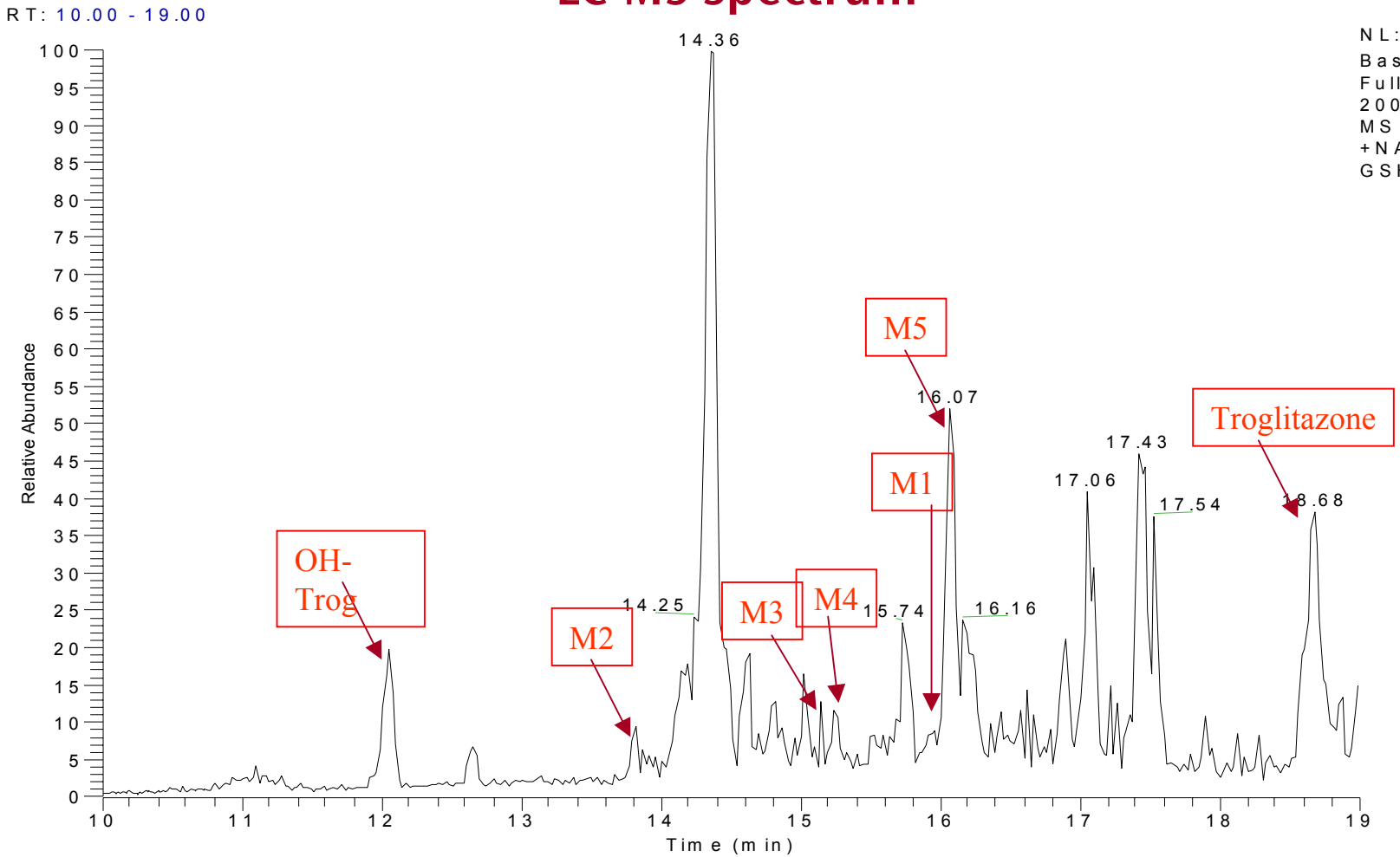




-*Glutathione-adduct conjugation (LC-MS-MS).*

Troglitazone and GSH conjugates

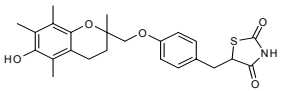
LC-MS Spectrum

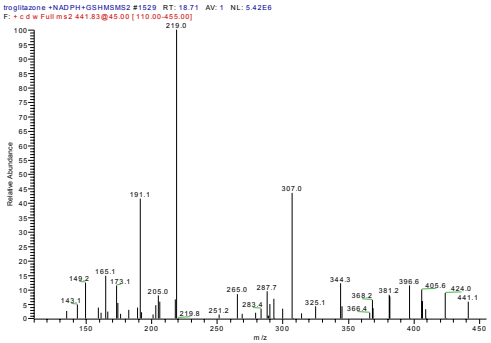


Troglitazone and GSH conjugates

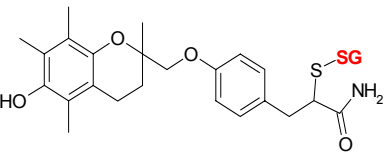
LC-MS/MS Spectrum

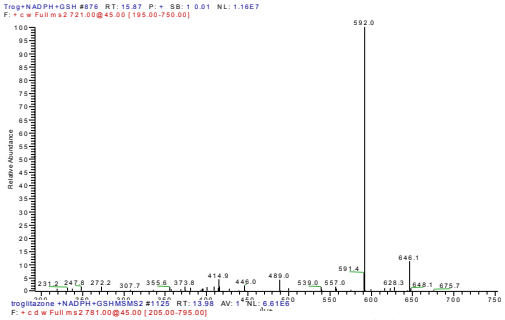
Troglitazone



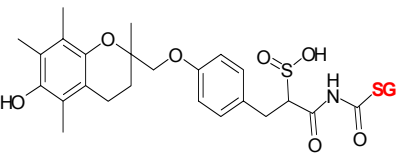


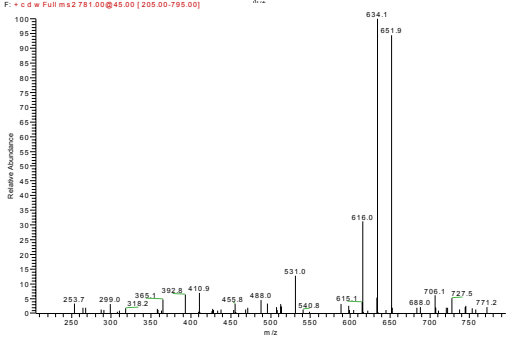
M1



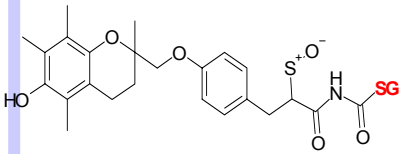


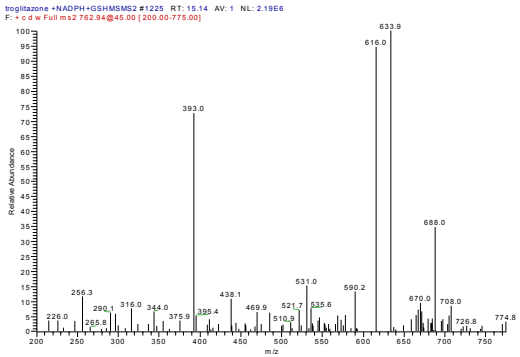
M2



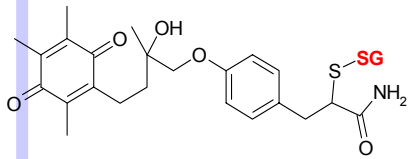


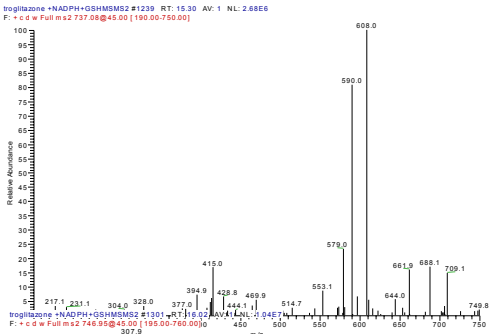
M3



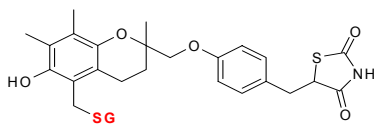


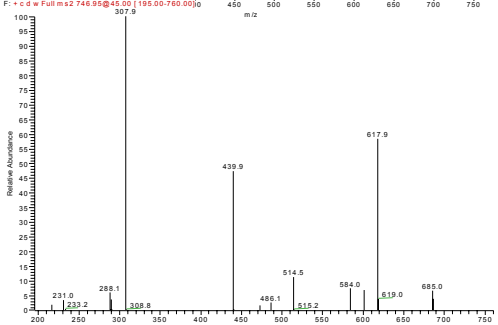
M4





M5





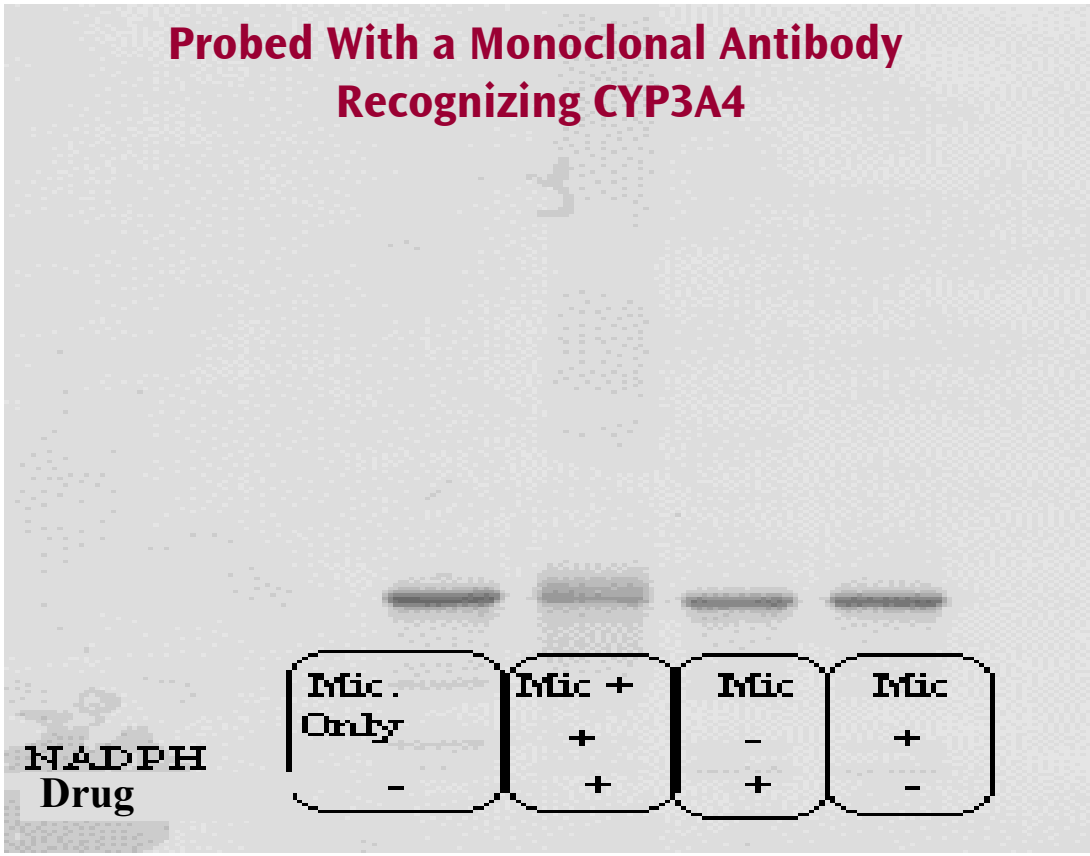
Group sanofi aventis
Because health matters

Mechanism-Based Inhibition of CYP 3A4 (eg. Human)

Procedure

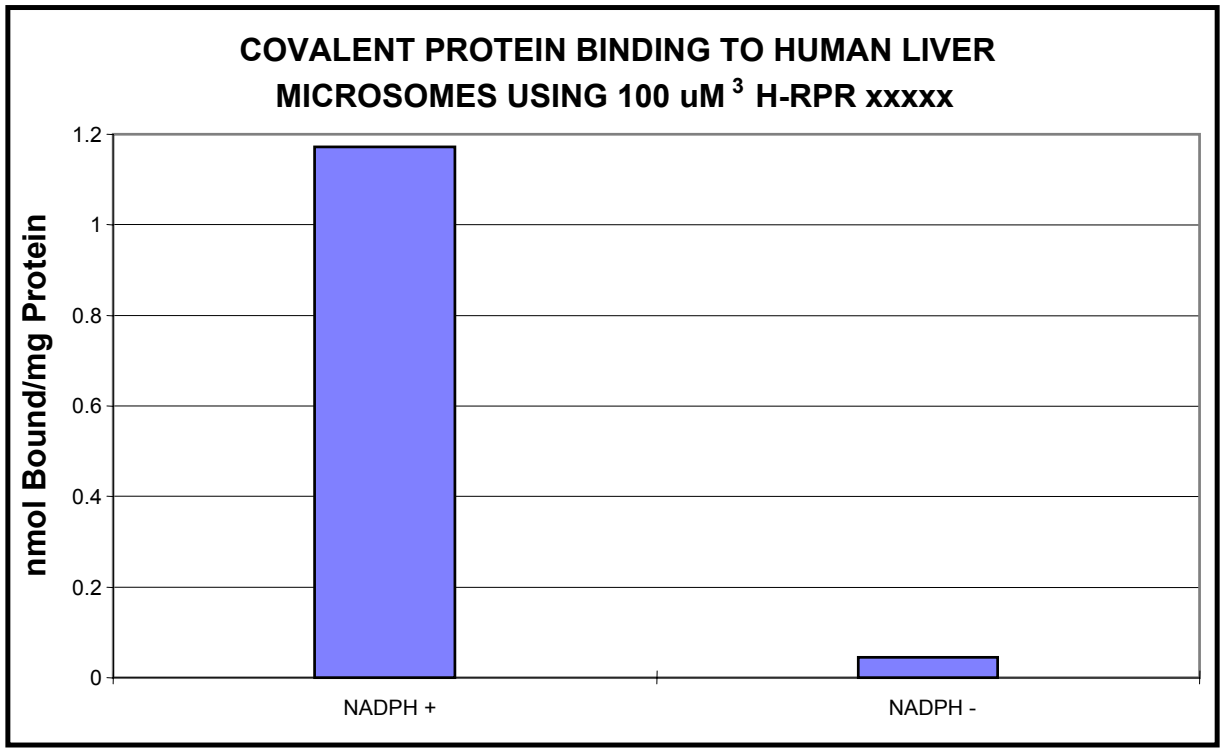
- Incubation contained 0.15 mg of pooled human liver microsomes
- Test article was incubated W or W/O NADPH.
- After 10 min of incubation the samples were centrifuged 30 min @ 100,000*g* and washed with PBS 3X.
- Western blot probed with antibody for CYP 3A4

CYP3A4 Inhibition in Human Hepatocytes



Human liver microsomes were incubated as mentioned in Methods and the blot was probed with a monoclonal antibody h333 (1 µg/ml) prepared against human liver cytochrome P450 3A4. The HRP labeled goat anti-mouse secondary antibody was used at a 1:1000 dilution.

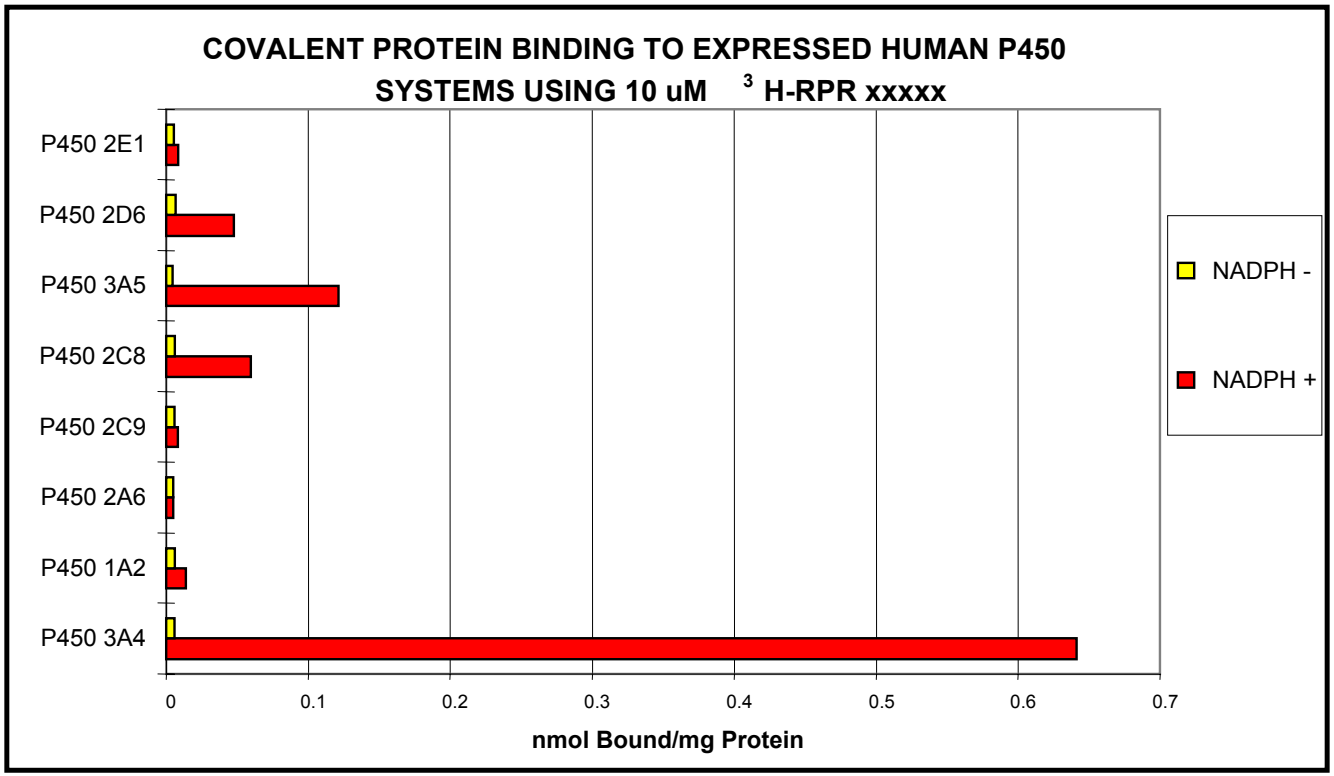
PROTEIN COVALENT BINDING OF ^{3}H-RPR xxxxx TO HUMAN LIVER MICROSOMES



Human liver microsomes were incubated with 10 µ M ^{3}H-RPR xxxxx with or without NADPH for 60 minutes at 37 ° C. The proteins were precipitated and washed with acetone to eliminate non-specific binding. The proteins were solubilized in 1% SDS and radioactivity was measured.

.

PROTEIN COVALENT BINDING OF ^{3}H-RPR xxxxx TO CYP ISOZYMES



Expressed human P450 isozymes were incubated with 10 µ M 3H-RPR xxxxx with or without NADPH for 60 minutes at 37 ° C. The proteins were precipitated and washed with acetone to eliminate non-specific binding. The proteins were solubilized in 1% SDS and radioactivity was measured.